REMARKS

Claims 1-4, 10-19, 26, 30-32 are currently pending in the application. Claims 5-8, 20-25, 27-29 and 33-35 are canceled as they are directed to a non-elected invention. Claim 9 is also cancelled, as it is now redundant in view of the amendments to claim 1. Claims 1-2, 4, 10-12, 18 and 30-32 are amended. New claims 36-49 are added.

The amendments and new claims find support in the specification. Electrosensitization after loading is supported in the specification, particularly at page 3, lines 8-10; page 3, line 26 to page 4, line 4; page 4, lines 23-25; page 5, lines 19-22; page 6, lines 11-17; page 44, lines 20-22; and page 45, lines 9-11 and 12-14. Performing electrosensitizing before loading is supported at page 3, lines 8-10; and page 3, line 26 to page 4, line 4. Use of multiple electrical pulses in electrosensitization is described throughout the specification, *e.g.*, at page 12, line 5-7; page 13, lines 14-20 and 23-25; page 14, lines 4-6; page 33, lines 9-11; page 36, lines 1-6; page 44, lines 17-18 and page 45, lines 12-14. Use of pulses in an exponential wave form is supported at page 13, lines 21-25; page 32, lines 21-22; page 33, lines 3-5; and page 37, lines 23-25, use of a square wave form is described at page 12, line 30 to page 13, line 2; page 13, lines 21-25; page 32, lines 21-22; and page 38, lines 1-2, and use of a modulated wave form is supported at page 13, lines 21-25.

Use of osmotic shock to load the cell is described at page 16, lines 3-8 and 19-21 and 24-26; page 32, line 23 to page 33, line 2; page 34, lines 5-6; page 35, line 13 to page 36, line 5; page 37, lines 10-18; page 38, lines 6-15; page 40, lines 1-4, 9-10 and 23-26; page 41, Table 1, to page 42, line 2; page 44, lines 18-20; page 45, lines 12-14 and 26-29. Support for the use of hypotonic dialysis can be found at page 4, lines 17-18; page 10, lines 5-11; page 16, lines 3-8, 19-21 and 24-26; page 16, line 27 to page 17, line 25; and page 18, lines 9-10, and hypoosmotic dialysis is discussed at page 10, lines 5-11; and page 16, lines 24-26.

Loading of multiple agents into a cell is discussed at page 15, lines 14-15 and 20-22; page 16, lines 19-21; page 18, lines 19-21; page 19, lines 8-10; page 24, line 27 to page 25, line 7; and page 26, lines 10-21. Support for re-sealing of cell after loading can be found at page 17,

Claims 10-12 have been amended to delete the dependency to claim 5, a non-elected claim. Claim 32 has been amended to spell out "polyethylene glycol (PEG)".

Claim 4, however, has not been amended. Applicants are making a *bona fide* attempt to address the objection, but are genuinely confused by the objection. Claim 4 as filed is an independent claim, and was placed in Group I with claims 1-3 in the Restriction Requirement. Should the objection be maintained, Applicants respectfully request clarification as to how this claim comprises non-elected subject matter.

Reconsideration and withdrawal of the objections is respectfully requested.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 9-19, 29 and 30-32 are rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification, while being enabling for pre-sensitizing red blood cells with electroporation *in vitro*, does not reasonably provide enablement for presensitizing the red blood cells with electroporation *in vivo*, or presensitizing using ultrasound. The Office Action concludes that the specification does not enable a person skilled in the art to practice the invention commensurate in scope with the claims.

Specifically, the Office Action states the specification is "silent with regard to an in vivo loading process, how to apply an electric field selectively on the population of red blood cells *in vivo* and how to [deliver] the agent to be loaded into the particular cells".

The claims do not recite *in vivo* pre-sensitization. Rather, claim 9 recited pre-sensitizing or electrosensitizing *in vitro* or *ex vivo*. Claims 1 and 4 have been amended to recite that the presensitization and electrosensitization are done *in vitro* or *ex vivo*. Claim 9 has been cancelled as redundant in view of the amendments to claim 1. Claim 30, which is similar in structure to claims 1 and 4, has been amended similarly.

Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

lines 5-7; page 18, lines 24-29; page 34, lines 23-27; page 35, lines 28-30; page 36, line 2; and page 38, lines 16-28.

New claims 43-47 are supported by claim 18 as originally filed, and also by the specification at page 14, line 24 (claim 43), line 25 (claim 44), line 26 (claim 45) and line 28 (claims 46 and 47).

Basis for the amendments to the specification at page 9 can be found at Fig. 6 as filed and in the discussion on page 40, lines 15-18, where the meanings of the symbols are clearly identified. Similarly, basis for the amendments at pages 42-45 can be found at pages 9 and 10 as filed, where Fig. 8 is clearly identified as showing release of antibody, Fig. 9 as showing release of β-galactosidase, and Fig. 10 as showing release of oligonucleotid. It is therefore clear that the Figures should illustrate the results in sections I, II and III, respectively, of Example 7.

No new matter is added.

Formal Drawings

Formal drawings are filed concurrently with this Amendment.

Priority

The specification has been amended to include specific reference to the applications from which the present application claims priority. These priority claims were originally presented in the application transmittal papers.

A certified copy of PCT/GB00/03056 is filed concurrently herewith.

Claim Objections

The Office Action states that claims 4 and 9-12 are objected to because they comprise subject matter that reads on a non-elected invention, and claim 32 is objected to because the abbreviation "PEG" should be spelled out in the claim.

Claim 9 has been canceled, mooting the rejection to this claim.

The Office Action also states that the claims embrace presensitizing the red blood cells with an ultrasound wave energy, but that the specification only teaches using ultrasound for disruption of the red blood cells.

Applicants respectfully traverse. The specification teaches the use of ultrasound, or sonoporation, for presensitization at page 11, lines 12-13 and in the section titled "Presensitization Using Ultrasound", at page 14, line 11 to page 15, line 12. In these sections, several different energy levels are suggested for use. In addition, Example 8 presents data showing that ultrasound can replace electricity as a pre-sensitization step (see, *e.g.*, lines 22-25 of page 45 ("It was therefore of interest to determine whether or not exposure of erythroyctes to ultrasound prior to hypoosmotic loading would contribute positively to loading of those cells and if so, could those cells be rendered sensitive to ultrasound by subsequently exposing them to sensitising electric pulses.")). Page 46, lines 21-27 shows that exposure to ultrasound prior to loading by dialysis enables 93% of the cells to be loaded with the agent ("When cells were loaded using the ES+HD+ES protocol, a major peak shifted to the right was detected and this indicated that almost all (88%) of the cells in the population were maximally loaded (Figure 11). When the protocol employing a sonoporative pre-sensitisation step prior to hypotonic dialysis was analysed on flow cytometry it was found that again, most of the cells resided in a peak shifted well to the right in Figure 11. This again indicated that almost all of the cells (93%) were loaded with fluorescent antibody."). Similar results are presented in Table 2 on page 47. Therefore, the specification fully discloses and supports presensitization by ultrasound. One of ordinary skill can easily practice the invention as claimed, without undue experimentation.

Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 2, 18 and 19 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Specifically, the Office Action states that claim 2 is indefinite because the claim is difficult to comprehend. This claim has been amended to recite that step (c) comprises loading the red blood cell with a first agent and a second agent.

Claim 18 is also rejected as indefinite, the Office Action stating that the specification fails to define the meaning of "diagnostic ultrasound" or "therapeutic ultrasound".

Applicants respectfully traverse the rejection. "Diagnostic ultrasound" is defined in the specification at page 14, lines 22-25 as generally being up to 100 mW/cm^2, sometimes as much as 750 mW/cm^2 ("When used as a diagnostic tool ("diagnostic ultrasound"), ultrasound is typically used in an energy density range of up to about 100 mW/cm^2 (FDA recommendation), although energy densities of up to 750mW/cm^2 have been used."). Likewise, ultrasound as used in therapy is defined in the specification (at page 14, lines 25-28) as being of a much higher energy level, generally being 3-4 W/cm^2, or as much as 100 W/cm^2, or even as high as 1 kW/cm^2 or higher ("In physiotherapy, ultrasound is typically used as an energy source in a range up to about 3 to 4 W/cm^2 (WHO recommendation). In other therapeutic applications, higher intensities of ultrasound may be employed, for example, HIFU at 100W/cm^2 up to 1kW/cm^2 (or even higher) for short periods of time."). Applicants also note that the specification teaches throughout that to accomplish a given result, higher energy levels can be used for shorter periods of time, or that lower energy levels can be used for longer period of time (see, *e.g.*, page 15, lines 11-12).

Applicants therefore respectfully submit that when read in light of the specification, claim 18 is clear on its face.

Claim 19 is also rejected, the Office Action stating that the limitation of ultrasound power energy by power level renders this claim indefinite because (1) it is unclear what kind of power the claim embraces ("electric current or some other power source"), and (2) different sonicators may generate different ultrasound wave frequencies.

With regard to the kind of power the claim embraces, claim 19 as written refers to the output of the device as being from about 0.05 W/cm^2 to about 100 W/cm^2. The claim cannot be interpreted any other way, because claim 19 depends from claim 4, which recites that the ultrasound is applied to the red blood cell. The cells are therefore exposed to ultrasound at a power level of about 0.05 W/cm^2 to about 100 W/cm^2.

The intensity, or "action strength" of ultrasound power output is expressed in terms of rate of energy delivered per unit area. The output of a particular machine is expressed in Watts divided by the effective radiating surface area (ERA) of the ultrasound applicator. If the transducer's output is 3 W/cm^2, than a head with an ERA of 10 cm^2 results in an output of 3 W/cm^2 (30 W per 10 cm^2 = 3 W/cm^2). If a 5 cm^2 head is attached instead, then the output will be 6 W/cm^2 (30 W per 5 cm^2 = 6 W/cm^2).

Recitation in claim 19 of the intensity of the ultrasound therefore controls for the fact that different machines have different output levels, and renders the claim clearer in view of the number of different ultrasound machines available.

Applicants therefore respectfully submit that the claim is clear as written, and request that the rejection on this basis be reconsidered and withdrawn.

In view of the arguments and amendments discussed above, reconsideration and withdrawal of the rejection of claims 2, 18 and 19 under 35 U.S.C. § 112, second paragraph is respectfully requested.

Claim Rejections Under 35 U.S.C. §§ 102(e), (f)

Claims 4, 18 and 19 are provisionally rejected under 35 U.S.C. § 102(e) as being anticipated by claims 8-14 of commonly assigned co-pending Application No. 09/748,063. Claims 4, 18 and 19 are also rejected under 35 U.C.S. § 102(f) on the grounds that Applicants did not invent the claimed subject matter, the Office Action stating that co-pending Application No. 09/748,063 has a different inventive entity than the present application.

The present application is a continuation-in-part of International Application No. PCT/GB00/03056, filed on August 9, 2000, and also claims the benefit of U.S. Provisional Application No. 60/181,796, filed on February 11, 2000, and United Kingdom Application No. 0002856.3, filed February 8, 2000. In addition, the present application and U.S. App. No. 09/748,063 are both assigned to Gendel, Ltd., and the claimed subject matter of both applications was invented by persons who, at the time of filing of the applications, were obligated to assign to Gendel, Ltd.

According to the Manual of Patent Examining Procedure (MPEP) § 2137.01:

> "[A] joint application or patent and a sole application or patent by one of the joint inventors are [by] different legal entities and accordingly, the issuance of the earlier filed application as a patent becomes a reference for everything it discloses" (*Ex parte Utschig*, 156 USPQ 156, 157 (Bd. App. 1966)) except where:
>
> (A) the claimed invention in a later filed application is entitled to the benefit of an earlier filed application under 35 U.S.C. 120 (an overlap of inventors rather than an identical inventive entity is permissible). In this situation, a rejection under 35 U.S.C. 102(e) is precluded. See *Applied Materials Inc. v. Gemini Research Corp.*, 835 F.2d 279, 281, 15 USPQ2d 1816, 1818 (Fed. Cir. 1988) ("The fact that an application has named a different inventive entity than a patent does not necessarily make that patent prior art."); and
>
> (B) the subject matter developed by another person and the claimed subject matter were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person. In this situation, a rejection under 35 U.S.C. 102(f)/103 or 102(g)/103, or 102(e)/103 for applications filed on or after November 29, 1999, is precluded by 35 U.S.C. 103(c). See MPEP § 706.02(l) and § 706.02(l)(1).

(emphasis added). The present application is entitled to the benefit of an earlier filed application under 35 U.S.C. 120, and both U.S. App. No. 09/748,063 and the present application are owned by the same entity. U.S. App. No. 09/748,063 is therefore not prior art under 35 U.S.C. § 102(e) or (f), and Applicants request that the rejection on this basis be reconsidered and withdrawn.

Claims 1, 3, 9-10, 14-17, 26 and 30-31 are also rejected under 35 U.S.C. § 102(f), on the grounds that Applicants did not invent the claimed subject matter, the Office Action stating that co-pending Application No. 09/779,186 has a different inventive entity than the present application.

Like U.S. App. No. 09/748, 063, above, the present application claims benefit of an earlier filing date under 35 U.S.C. § 120, and the subject matter of the present application and U.S. App. No. 09/779,186 are both assigned to Gendel, Ltd.

U.S. App. No. 09/779,186 is therefore not prior art under 35 U.S.C. § 102(f), and Applicants request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 102(b)

Claims 1, 9 and 10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Mouneimne *et al.* (U.S. Pat. No. 5,236,835), claims 1-2 and 9-10 are rejected as anticipated by Lizano *et al.* (*Biochem. Biophys. Acta* 1425:328-336, 1998), claims 1-2, 4, 9-10 and 18 are rejected as anticipated by Mitchell *et al.* (*Biotech. Appl. Biochem.* 12:264-75, 1990), and 1, 9-10 and 12 are rejected as anticipated by Zimmermann *et al.* (U.S. Pat. No. 4,289,756).

Claims 1 and 10 Are Novel Over Mouneimne et al.

Claims 1, 9 and 10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Mouneimne *et al.* (U.S. Pat. No. 5,236,835). The Office Action states that Mouneimne *et al.* teaches a method of incorporating CD4 or a glycophorin into a red blood cell by application of an electric field in a solution containing the CD4 or glycophorin, and that this reference therefore anticipates these claims. Claim 9 has been canceled.

Mouneimne *et al.* teaches a method of incorporating CD4 or glycophorin into a red blood cell by "exposing the red blood cell to an electric field while the cell is suspended in an electro-insertion medium in the presence of a buffered solution (suspension) of the proteins" (abstract). This reference therefore teaches electroporation as it is generally performed in the prior art.

In contrast, amended claim 1 recites a method of producing a red blood cell comprising an agent, where the method comprises two discrete steps: (a) pre-sensitizing the red blood cell *in vitro* or *ex vivo*, followed by (b) loading the red blood cell with an agent. The steps of pre-sensitizing and loading clearly take place in two steps: a first step, followed by a second step.

In contrast, Mouneimne *et al.* teaches only a single step. This references teaches electric field pulse loading, in which the cells must be suspended in a solution containing the agent to be loaded, so that the agent can enter the cells by passing through the pores transiently created during the electric pulse. In electroporation as described in Mouneimne *et al.*, the two steps of pre-sensitization and loading cannot be separated. The teachings of the Mouneimne *et al.* reference therefore do not anticipate the claimed methods, and Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claims 1, 2 and 10 Are Novel Over Lizano et al.

Claims 1, 2, 9 and 10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Lizano *et al.* (*Biochem. Biophys. Acta* 1425:328-336, 1998). Lizano *et al.* is stated to teach a method of encapsulating two types of enzymes (ADH and ALDH) into red blood cells by electroporating the red blood cell-enzyme mixture. Claim 9 has been canceled.

Like the Mouneimne *et al.* reference discussed above, Lizano *et al.* does not teach a first step followed by a second step, but rather, teaches electric field pulse loading by suspending the cells in a solution containing the agent while they are subjected to an electric pulse. There is therefore no discrete pre-sensitization or sensitization step, as the agents enter the cell through the pores transiently created during the electric pulse.

Lizano *et al.* fails to teach any kind of pre-sensitization or sensitization step that is separate from the loading step, and therefore fails to anticipate the claims as amended. Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claims 1-2, 4, 10 and 18 Are Novel Over Mitchell et al.

Claims 1, 2, 4, 9, 10 and 18 are rejected under 35 U.S.C. § 102(b) as being anticipated by Mitchell *et al.* (*Biotech. Appl. Biochem.* 12:264-75, 1990), as evidenced by Halaka (U.S. Pat. No. 6,071,480). The Office Action states that Mitchell *et al.* teaches a method of encapsulating rIL-2 and human serum albumin into red blood cells by applying an electric field pulse. The cells are then lysed by sonication or hypotonically.

Like Mouneimne *et al.* and Lizano *et al.*, Mitchell *et al.* fails to create cells loaded with an agent by separate steps of sensitization and loading, but instead uses electroporation, which loads the cells with an agent through pores transiently created during exposure to an electric pulse. Mitchell *et al.* does not teach a first step followed by a second step, and therefore fails to anticipate Applicants' amended claims, and the reconsideration and withdrawal of the rejection on this basis is respectfully requested.

Claims 1, 10 and 12 Are Novel Over Zimmermann et al.

Claims 1, 9, 10 and 12 are rejected under 35 U.S.C. § 102(b) as being anticipated by Zimmermann *et al.* (U.S. Pat. No. 4,289,756). The Office Action states that Zimmermann *et al.* teaches a method of introducing a medicament (such as a protein) into cells (including red blood cells) by using a combination of osmotic pressure and an electric field. The Office Action also states that Applicants' claim 12 "is drawn to a method of loading RBCs using [a] combination of hypotonic dialysis and electroporation."

Applicants respectfully traverse this interpretation of the claims. Amended claim 12 depends from amended claim 1, which recites two discrete steps: (a) pre-sensitizing the red blood cell *in vitro* or *ex vivo*, followed by (b) loading the red blood cell with an agent. Claim 12 adds the limitation that the loading is done by hypotonic dialysis. Claim 12, if written in independent form, would not teach a combination of hypotonic dialysis and electroporation, but would teach the discrete steps of: (a) pre-sensitizing the red blood cell *in vitro* or *ex vivo*, followed by (b) loading the red blood cell with an agent by hypotonic dialysis.

The preamble of claim 1 of Zimmermann *et al.* describes methods of "increasing the permeability of the cell membranes by the effect of osmotic pressure or by the effect of an electric field, or both", and teach that the agent to be loaded can be a protein. However, this reference does not teach a first step of sensitization followed by a second step of loading, and so cannot anticipate Applicants' claims. The rejection on the basis of this reference should be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 1, 3, 13-17, 26, and 30-31 are rejected under 35 U.S.C. § 103 as being unpatentable over Mitchell *et al.* (*Biotech. Appl. Biochem.* 12:264-75, 1990) in view of Ortiz *et al.* (*Mut. Res.* 327:161-9, 1995).

The Office Action states that Mitchell *et al.* teaches that loading two proteins into red blood cells by electroporation and the release of red cells are positively linked to increased pulse length, frequency, and intensity of the electric field, and that this reference teaches using an electric field ranging from 6-8 kv/cm from 5-40 μs, but that Mitchell *et al.* does not use multiple doses of electroporation.

The Office Action also states that Ortiz *et al.* teaches a method of loading CHO cells with two enzymes using different combinations of single and double doses of electroporation, and teaches that when cells have been electroporated once, they can resist a second electroporation without significant loss of cell viability.

From this, the Office Action concludes that it is known that electroporation enhances protein loading in red blood cells, and that two agents can be loaded together with two doses of electroporation without significant loss of viability.

The Mitchell *et al.* reference is discussed above, where it is pointed out that Mitchell *et al.* does not teach separate steps of sensitization and loading.

Ortiz *et al.* also does not teach a first step of pre-sensitization and a second step of loading. Rather, Ortiz *et al.* disclose single and double electroporation experiments, and state that "CHO cells are able to resist two electroporations given 60 min apart". Like the other

references cited in the Office Action, however, Ortiz *et al.* disclose the use of electroporation, in which substances enter cells through pores in the cell membrane transiently created by a pulse of electricity. The electrical treatment and the permeation occur as a single step, and are not done separately. The combination of Mitchell *et al.* and Ortiz *et al.* therefore produces multiple rounds of electroporation, but not a first step of pre-sensitization, followed by a second step of loading, as recited in Applicants' claims.

In contrast, Applicants' specification teaches that treatment with an electric field increases the cells' sensitivity to ultrasound-mediated disruption, resulting in the ability to use lower doses of ultrasound, with less possibility of damage to normal cells in the body (see, *e.g.*, page 2, line 27 to page 3, line 2). If the cells are pre-sensitized before loading, then up to 100% loading efficiency can be achieved (see, *e.g.*, page 3, lines 3-5). However, loading by dialysis can also reduce the sensitivity of the cells to ultrasound disruption, but sensitivity can be restored by application of another sensitization step after loading is completed (see, *e.g.*, page 3, lines 7-10 and page 10, lines 26-27).

The combination of Mitchell *et al.* and Ortiz *et al.* to use multiple rounds of electroporation cannot render obvious the use of lower doses of ultrasound to release the agent(s) due to the increased sensitivity of cells produced by Applicants' claimed methods. The combination also does not render obvious the result of increased loading efficiency after sensitization.

The specification also teaches that the pre-sensitization and loading steps can be separated to produce kits of pre-sensitized cells ready to be loaded. The combination of Miller *et al.* and Ortiz *et al.* neither teaches nor suggests that electroporated cells are pre-sensitized and can be loaded with an agent at a later time, that is, the combination fails to teach or suggest a first step of pre-sensitization followed by a second step of loading.

The advantages of the present invention are (1) the ability to use lower doses of ultrasound to disrupt the loaded cells, thereby reducing damage caused to normal cells and tissues caused by higher doses of ultrasound, (2) increased efficiency of cell loading with the

agent(s), and (3) the ability to produce kits containing pre-sentitized cells that can be loaded at a later time.

The cited references do not teach or suggest any of these advantages. The combination of these references therefore fails to render obvious the claimed subject matter, and Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claims 1, 3, 13-17, 26 and 30-32 are rejected under 35 U.S.C. § 103 as being unpatentable over Mitchell *et al.* (*Biotech. Appl. Biochem.* 12:264-75, 1990) and Ortiz *et al.* (*Mut. Res.* 327:161-9, 1995), and further in view of Magnani *et al.* (U.S. Pat. No. 6,139,836).

Mitchell *et al.* and Ortiz *et al.* are applied as for claims 1, 3, 13-17, 26, and 30-31, and Magnani *et al.* is added because it teaches using PEG for "in vivo delivering loaded erythrocytes", the Office Action contending that it would therefore have been obvious to use PEG as a pharmaceutical carrier.

However, as discussed above, Applicants' claims are not obvious in view of the combination of Mitchell *et al.* and Ortiz *et al.* , because the combination fails to teach or suggest a first step of pre-sensitization followed by a second step of loading. The addition of Magnani *et al.*, while teaching PEG as a carrier, fails to teach the basic advantages (as discussed above) to be had in the separation of the steps of sensitization and loading of cells. The combination of the three references fails to produce or suggest the advantages of Applicants' invention.

The rejection on the basis of the combination of these references should therefore be reconsidered and withdrawn.

Claims 1, 3-4, 13-17, 19, 26 and 30-31 are rejected under 35 U.S.C. § 103 as being unpatentable over Mitchell *et al.* (*Biotech. Appl. Biochem.* 12:264-75, 1990) and Ortiz *et al.* (*Mut. Res.* 327:161-9, 1995) as applied above, and further in view of Halaka (U.S. Pat. No. 6,071,480). The Office Action states that Halaka teaches using different power levels to generate different ultrasonic wave frequencies, such as 100 W power, and that it would therefore

have been obvious to use this power level in combination with the methods of Mitchell *et al.* and Ortiz *et al.* to practice Applicants' invention.

The disclosure of particular ultrasound power levels in Halaka does not address the deficiencies of the Mitchell *et al.*/Ortiz *et al.* combination, as discussed above. The combination of the three cited references fails to teach any separation of sensitization and loading steps, or the advantages of doing so.

Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

Double Patenting

Claims 1, 3, 9-10, 13-17, 26 and 30-31 are rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 19-21 of U.S. App. No. 09/779,186. Claims 4 and 18-19 are also rejected as being unpatentable over claims 8-14 of U.S. App. No. 09/748,063, and claims 1, 3, 9-10, 12-17, 26 and 30-31 are rejected as unpatentable over claims 26 and 29-37 of U.S. App. No. 09/779,188.

Applicants will consider the filing of a Terminal Disclaimer upon an indication of allowable subject matter.

MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please add the following paragraph at page 1, between lines 2 and 3, before "FIELD OF THE INVENTION":

RELATED APPLICATIONS

This application is a continuation-in-part of International Application No. PCT/GB00/03056, which designated the United States and was filed on August 9, 2000, and was published in English, which claims the benefit of U.S. Provisional Application No. 60/181,796, filed on February 11, 2000, and also United Kingdom Application No. 0002856.3, filed February 8, 2000.

Please replace the paragraph at page 9, lines 5-12, with the following paragraph, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Figure 6 shows the stability of cellular integrity (cell numbers) and ultrasound sensitivity during storage at 4°C. Cells were loaded with FITC labelled antibody using a process comprising pre-sensitisation/hypotonic dialysis/electrosensitisation (ES-HD-ES) and at the indicated times cell numbers (▲) were determined by direct counting. The percentage of cells that lysed following exposure to ultrasound was also determined (♦) for each sample. The X-axis represents the time in days, the left Y-axis represents the percentage of cells remaining intact and the right Y-axis represents the percentage lysis observed following exposure to ultrasound.

Please replace the paragraph running from page 42, line 20 to page 43, line 3, with the following paragraph, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

In the loading and sensitisation protocol, cells were loaded at a concentration of 1.1mg of antibody per ml of packed cell volume (PCV). 0.1ml aliquots of cells at $7x10^8$ cells/ml were exposed to ultrasound at intensities shown in Figure 8[7] and samples were analysed for cell lysis by direct counting. In addition, the amount of antibody released following treatment with ultrasound was determined by ELISA analysis of cell supernatants harvested following centrifugation. The results obtained are shown in Figure 8[7] and they demonstrate that cells were preferentially lysed at ultrasound power densities greater than $2W/cm^2$. Control cells exhibited little or no effect when treated with ultrasound at these power densities. In addition, at and above $2W/cm^2$ antibody payload was detected in supernatants harvested following ultrasound treatment. In addition, when the total amount of antibody released from the cells using ultrasound was compared with that released following hypotonic lysis in 0.01% (v/v) Triton X100 it was found that 77% of the total antibody was released in the former. The remainder could be found in debris that was recovered by centrifugation following ultrasound treatment.

Please replace the paragraph running from page 43, line 28 to page 44, line 8, with the following paragraph, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

In these experiments loaded cells contained approximately 1mg of enzyme per ml of packed cell volume. The results obtained following treatment of these preparations with ultrasound are shown in Figure 9[8]. Samples were treated at the indicated power

densities as shown and samples were analysed for cell lysis by cell counting. Lysis increased with increasing power density up to a maximum at about 3W/cm^2. Exposure of control normal cells to similar ultrasound conditions had little or no effect on cell lysis and this was confirmed by the absence of haemoglobin in supernatants following removal of cells by centrifugation. When supernatants were harvested by centrifugation, following exposure of the sensitised and loaded cells to ultrasound and analysed for enzyme content, it was found that increasing amounts of enzyme were released with increasing power density up to a maximum at 3W/cm^2.

Please replace the paragraph on page 45, lines 1-6, with the following paragraph, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

The results obtained following treatment of these loaded preparations with ultrasound are shown in Figure 10[9]. As with the above two examples, cell lysis of the sensitised and loaded preparation occurs between 2 and 3 W/cm^2. Under these ultrasound conditions there is little or no effect on control erythroyctes. In addition oligonucleotide begins to appear in harvested supernatants between 2 and 3 W/cm^2 demonstrating ultrasound-mediated release of oligonucleotide payload from the vehicle.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 1-2, 4, 10-12, and 30-32 as follows:

1. (Amended) A method of producing a red blood cell comprising an agent the method comprising: a first step of pre-sensitizing a red blood cell *in vitro* or *ex vivo*, followed by a second step of loading said red blood cell with an agent.
   [(a) providing a said red blood cell;
   (b) pre-sensitising said red blood cell; and,
   (c) loading said red blood cell with said agent.]

2. (Amended) A method according to claim 1, wherein said second step [(c)] comprises loading [comprising leading a first and] said red blood cell with a first agent and a second agent.

4. (Amended) A method for selectively releasing an agent from a red blood cell comprising the steps of:
   (a) pre-sensitising a red blood cell *in vitro* or *ex vivo*;
   (b) loading said red blood cell with an agent;
   (c) electrosensitising said red bood cell *in vitro* or *ex vivo*; and
   (d) effectuating substantial release of said agent from said sensitised red blood cell by applying ultrasound[.] at a frequency and energy sufficient to cause disruption of sensitized [unsensitised] red blood cells[,].

10. (Amended) A method according to claim 1 [or claim 5], wherein said pre-sensitising step comprises applying an electric field to said red blood cell.

11. (Amended) A method according to claim [claims] 1 [or claim 5], wherein said

pre-sensitising step further comprises applying ultrasound to the red blood cell.

12. (Amended) A method according to claim 1 [or claim 5], wherein said loading step comprises hypotonic dialysis.

30. (Amended) A pharmaceutical composition comprising a red blood cell composition made by a process comprising:

[(a) providing a red blood cell;]

(a[b]) pre-sensitizing a [said] red blood cell *in vitro* or *ex vivo*; and

(b[c]) loading said red blood cell with an agent[; and]

[(d) electrosensitizing said red blood cell].

31. (Amended) The composition of claim 30 [31] wherein said red blood cell composition further comprises a red blood cell is immunocompatible in a vertebrate.

32. (Amended) The composition of claim 31 wherein said red blood cell comprises polyethylene glycol (PEG).

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the reconsideration and withdrawal of the rejections over the claims of the present invention.

Respectfully submitted,

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